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Previews

Multiple Personalities in the Ventral Tegmental Area

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A small number of ventral tegmental area dopamine neurons engage in numerous and apparently contradictory functions—how can this be? A clue is provided by Lammel and colleagues in this issue of *Neuron*: some VTA dopamine neurons display synaptic plasticity in response to cocaine, and others in response to pain, and these populations are distinguished by their axonal projections and Ih.

Dopamine (DA) transmission by ventral midbrain neurons plays fundamental roles in voluntary motor function, habit learning, and motivation, while degeneration or dysregulation of these neurons is associated with Parkinson's disease, schizophrenic psychosis, and drug addiction. How can a small number of neurons (300,000–600,000 in human, ~45,000 in rat; German and Manaye, 1993) be responsible for so much? A study in this issue of *Neuron* provides the latest chapter in the study of what is turning out to be a complex set of personalities within this group of neurons (Lammel et al., 2011).

Nearly in tandem with the discovery of DA in the CNS by Arvid Carlsson and colleagues, and prior to the use of immunocytochemistry to detect tyrosine hydroxylase (TH), which is involved in DA synthesis, Kjell Fuxe, Annica Dahlström, and colleagues, using the "Falck-Hillarp" fluorescent histochemical technique, produced remarkably detailed images of ventral midbrain DA cell bodies and their axonal projections. The DA neurons of the laterally located "A9" substantia nigra pars compacta (SNc) were shown to principally innervate the caudate putamen, an area important for sensorimotor integration and control: indeed, Carlsson conjectured that the loss of DA release from these neurons causes parkinsonism (Carlsson, 1959). The neighboring, medially located "A10" ventral tegmental area (VTA) neurons were found in these and subsequent tracer studies to project to comparatively divergent areas, including the nucleus accumbens (NAc), limbic regions, and cerebral cortex (Swanson, 1982). While individual SNc neurons send axon collaterals to multiple brain regions, axons arising from VTA neurons show minimal collateralization.

Reinforcing and Aversive Stimuli: Can both Enhance VTA DA Activity

As the characterization of the ventral midbrain DA neuron cell groups and projections proceeded in Europe, James Olds and colleagues clearly implicated the A10 neurons in the effects of addictive drugs, each of which has later been found to enhance synaptic DA levels by means that dissociate it from normal behavioral control, as well as reward-based learning. Most remarkably, in the Olds lab's series of intracranial self-stimulation studies, rats would press a lever thousands of times an hour to stimulate the projections of these neurons. But what promotes behaviorally and physiologically relevant activity of these neurons?

The Olds group recorded activity from VTA neurons and found that in a hungry animal, these neurons fire in response to a sound they had learned to associate with food, or in a thirsty animal, when presented with a sound associated with water. In contrast, playing sounds that were *not* associated with food to hungry animals could lower VTA neuronal activity. They suggested this indicated an "integration" of the state of an organism (i.e., hungry or thirsty) so that only a reward appropriate for that state would activate VTA neurons (Phillips and Olds, 1969). This initial insight and its descendents, including models of "motivational salience" and "reward-prediction-error" (Bromberg-Martin et al., 2010), have been spectacularly successful for predicting experimental results in behavioral studies.

Nevertheless, cracks in the edifice that VTA neuron activity simply reflects a confluence of reward and state appeared early and often (Bromberg-Martin et al., 2010). As recent examples, VTA DA neurons can respond to noxious stimuli with phasic excitation (Brischoux et al., 2009), while a social defeat protocol led to enhanced striatal DA release in the NAc measured by voltammetry (Anstrom et al., 2009).

Two obvious, nonexclusive possibilities could explain these discrepancies. One is that VTA DA neurons may receive different inputs, one set associated with reward and state, and another with aversive stimuli (Sesack and Grace, 2010). Alternatively, there may be multiple classes of VTA DA neurons that respond to particular stimuli in different ways. While the mystery remains unsolved, the present study may provide an important piece of the puzzle.

Clues for Multiple Personalities in the VTA

Lammel and colleagues in this and a preceding study (Lammel et al., 2008) have in part returned to approaches of the Swedish pioneers by characterizing ventral midbrain neurons by means of their terminal fields. In this case, rather

than adapting the Falck-Hillarp approach, they adapted an approach from Larry Katz and colleagues, injecting fluorescent beads into multiple axonal projection areas of ventral midbrain DA neurons, including the medial prefrontal cortex, the medial and lateral NAc, and the dorsal striatum. The fluorescent beads are endocytosed by axons and retrogradely transported to label cell bodies, and in this way neuronal cell bodies can be distinguished by their projection regions.

As expected from prior findings by Jochen Roeper (Neuhoff et al., 2002), an author of the present study, and Elyssa Margolis (Margolis et al., 2006a, 2006b), SNc neurons projecting to the dorsal striatum were mostly TH^+, while in the present study most posterior VTA projection neurons were also TH^+: the TH^- cells are likely GABAergic or glutamatergic rather than dopaminergic.

As in the Margolis study, the properties of the projection neurons sort by terminal field. TH^+ cells with pronounced Ih, due to hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, were in the SNc projecting to dorsal striatum and in the lateral VTA projecting to lateral NAc shell, while TH^+ cells of the medial posterior VTA projecting to the medial prefrontal cortex and medial NAc shell had no or very small Ih. These findings differ in part from those of Margolis (2006a, 2006b), which were in rat rather than mouse, and reported that all TH^+ neurons had some Ih, although some were very small.

Nevertheless, both studies should drive the field to reevaluate its understanding of VTA neurons, since the presence of a large Ih has been used to identify DA neurons in many previous studies. Thus, Ih^- VTA DA neurons that project to the prefrontal cortex and medial NAc, and are extremely important in behavior, have been relatively ignored in the literature (Margolis et al., 2006a).

One means to compare the synapses on the somatodendritic regions of these different VTA populations is to stimulate the region locally and measure the response to glutamate excitation with and without an NMDA antagonist. This provides an estimate of the fraction of excitation due to somatodendritic NMDA and non-NMDA, chiefly AMPA, receptors. The comparative responses are expressed as an AMPA to NMDA ratio, and an increase in fraction is generally interpreted as an increase in AMPA receptor signaling, assumed to reflect strengthening of excitatory synapses.

The previously ignored Ih^- medial VTA neurons that project to the medial prefrontal cortex and medial NAc shell had a higher basal AMPA to NMDA ratio than the better characterized Ih+ neurons, although it would be premature to draw the conclusion that this is due to a greater baseline level of excitatory input.

Differential Plasticity of VTA Neurons

Multiple studies of the AMPA to NMDA ratio at Ih+ VTA neurons show that changes can occur following a single injection of cocaine or one of a number of other addictive drugs—and these are generally thought to reflect rapid changes in the presence or makeup of AMPA receptors at glutamatergic synapses (Lüscher and Malenka, 2011). Might it be that relying on a large Ih to identify VTA DA neurons has led to a lack of investigation of other populations of VTA DA neurons, and therefore the field has been unaware of midbrain synaptic plasticity triggered by aversive stimuli?

To examine this issue, Lammel and colleagues administered an addictive drug (cocaine) or a painful stimulus (a shot of formalin to a paw) to mice. Note that neither of these was involved with a learning or reward-prediction-error mechanism; they were administered directly to the mice without pairing stimuli or training as in Olds's experiments. The cocaine would presumably act to enhance extrasynaptic DA levels by blocking reuptake by the DA transporter, while pain would presumably activate multiple CNS pathways.

As expected from previous results, Lammel et al. find that the AMPA to NMDA ratio of lateral VTA Ih+ neurons was increased by cocaine, while pain had no effect on those projecting to the NAc medial shell.

The responses of the previously uncharacterized VTA DA neurons that project to prefrontal cortex or medial NAc, however, were novel and surprising. The most robust plasticity response to cocaine, as manifested by the greatest increase in AMPA to NMDA with a long persistence (3 weeks), occurred in the DA neurons that project to the NAc medial shell.

Perhaps more surprising, Ih^- VTA DA neurons that project to the prefrontal cortex showed no cocaine-induced alteration of AMPA to NMDA ratio, but exhibited a robust increase with pain. In the case of this noxious stimulus, the duration of AMPA:NMDA alteration in mesocortical DA cells exhibited a comparatively transient increase, returning to baseline within 10 days. Intriguingly, the DA neurons projecting to the lateral NAc shell were affected by both stimuli, suggesting that neural signals about stimuli that are rewarding or aversive may converge in some cases onto the same DA neuron. Finally, the authors omitted amygdala-projecting VTA cells that they had previously examined from this study (Lammel et al., 2008), and there are additional projection areas that may have still more diversity in response.

Thus, within the VTA there are multiple populations of DA neurons defined by their cell body position, axonal projections, and HCN currents. They can exhibit synaptic plasticity driven by either cocaine or pain, which lends hints as to how these neurons respond to behaviorally relevant stimuli and possibly as to how aversive or rewarding stimuli might be differentially processed by VTA DA neurons.

Questions

The outstanding question of whether these changes are due to differential inputs or due to intrinsic properties of the neurons remains unanswered, as does the extent to which these mechanisms are involved in experience-dependent learning such as drug seeking.

It will be important to measure plasticity in response to more behaviorally relevant protocols that emulate learning in response to reward and aversion, perhaps incorporating optogenetic or other approaches to more clearly isolate particular inputs altered by stimuli. Further characterization of changes in the AMPA to NMDA ratio in Ih^- DA neurons is required to determine if these cells exhibit a change in the subunit composition of AMPA receptors (e.g., a switch to calcium-permeable GluA2-lacking receptors) that has been linked to drug-induced behavioral sensitization and conditioned place preference (Lüscher and Malenka, 2011).

A related issue is that while altered DA neurotransmission in the striatum and NAc is strongly implicated in various aspects of drug dependence, it is less clear if an altered AMPA to NMDA ratio as a form of plasticity plays a role. If the AMPA receptors are maximally induced by exposure to an addictive drug, would this occlude reward-related learning for the duration? It may be that the more complex alterations at corticostriatal synapses induced by these drugs lead to very long-term habits.

The finding that a drug that elevates DA transmission and is associated with reward or addiction (and pain, as a model of aversive stimuli) could involve analogous synaptic plasticity at different DA cells certainly will motivate new investigations. The excitatory input to the DA cells is extensive and involves glutamatergic afferents from the prefrontal cortex, superior colliculus, pedunculopontine tegmental nucleus, lateral dorsal tegmental nucleus, subthalamic nucleus, and additional areas (Sesack and Grace, 2010), and any of these could be responsible for differential responses of the VTA neurons. Moreover, there are multiple inhibitory and modulatory inputs and collaterals, and appropriate disinhibition or frequency-dependent filtering could play the key role in determining which inputs mediate this diverse plasticity.

Using anatomically rigorous techniques, Lammel et al. have now provided us a far more detailed roadmap of the VTA. Future studies of these neurons will need to take into account more precisely which DA neurons are examined, including whether a neuron expresses TH^+ and expresses Ih, with some idea of where the projections lie. As it is now relatively clear that some VTA DA neurons use glutamate as a cotransmitter (Hnasko et al., 2010), precisely which of them do so, and why?

Most promisingly, these findings suggest new means to determine more precisely which synapses regulate behavior. For example, would selective activation of Ih^- medial shell-projecting cells induce behavior associated with reward, addiction, or avoidance? This further dissection of the smaller and smaller groups of mouse VTA neurons—with only about 10,000 to start with!—seems likely to reveal the mysteries of the circuitry that control reinforcement-based learning that Olds and collaborators began to unveil 50 years ago.

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